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REFERENCES

- Haag, F., and Linkweiler, J. (1931) *Z. Immunforsch.*, **70**, 337.
Kahn, R. L. (1925) *Serumdiagnosis of Syphilis by Precipitation*, Baltimore.
— (1928) *The Kahn Test*, Baltimore.
Laughlen, G. F. (1935) *Canad. med. Ass. J.*, **33**, 179.
— (1938) *Canad. publ. Hlth J.*, **29**, 396.
Sachs, and Witebsky, E. (1928)¹ *Klin. Wschr.*, No. 26, p. 1233.
— (1929)² *ibid.* No. 5, p. 210.
— (1931)³ *ibid.* No. 43, p. 1993.

A RAPID MICROSCOPICAL METHOD FOR THE SERODIAGNOSIS OF SYPHILIS

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Rappaport and Eichhorn described in 1943 a rapid test for the serodiagnosis of syphilis. In trying out this test I noticed that great reliability and accuracy can be achieved in an even shorter time by using the microscope for the estimation. In fact I believe that this method (known in this hospital as the S.S. (Serodiagnosis of Syphilis method) is more reliable than is either the Kahn or Wassermann reaction.

Materials

The materials required are as follows.

- (1) Fresh beef heart antigen prepared with red-dyed mastic according to Rappaport and Eichhorn's method.
- (2) Diluting fluid (disodium hydrogen phosphate, 5 per cent weight in volume).
- (3) Glass capillary tubes of about 1 millimetre internal diameter and $4\frac{1}{2}$ inches long. (These can be prepared easily in the laboratory.)
- (4) S.S. tubes. These are prepared from glass tubing $\frac{1}{8}$ - $\frac{3}{8}$ inch in diameter, by cutting pieces $4\frac{1}{2}$ inches long, widening one end and sealing off the other.
- (5) Glass slides.
- (6) Glass square coverslips, No. 1.

Method

Preparation for microscopical examination.—Venous or capillary blood (or better still serum separated from the clot) is drawn into a capillary tube (as described in (3) above), so that three-quarters of the capillary are filled. The free end of the capillary, uncontaminated by blood or serum, is then sealed off in a flame, and the tube centrifuged for a short time until the cells are packed. After centrifuging, that part of the capillary which contains the packed cells is removed by cutting with a glass file.

One drop of the serum remaining in the capillary is introduced into the S.S. tube by tilting the capillary almost to the vertical position. (Cerebrospinal fluid is prepared according to the method described by Rappaport and Eichhorn, in two S.S. tubes.) To this drop of serum, a similar drop of antigen is added, the latter being vigorously shaken before use. The S.S. tube is now shaken for about $\frac{1}{2}$ minute, and finally a similar drop of the diluting fluid is added; the contents are then ready for microscopical examination, which can be carried out at any time within 24 hours.

Microscopical examination.—Immediately before examination, the contents of the S.S. tube are thoroughly shaken by tapping the bottom of the tube briskly about 10 times with the index finger.

One drop is poured out of the S.S. tube (gently to avoid air bubbles) on to a glass slide and covered with a microscopical glass square, No. 1. This wet preparation must be examined within 5 minutes, since drying may produce a false positive.

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It is advisable to use perfectly clean glass slides and coverslips, because impurities on the glass may imitate floccules and thus inadvertently a false positive reaction may be recorded. With some experience this danger is eliminated.

Interpretation of results

The "negative" is seen under the low power objective ($\frac{2}{3}$ inch) as a uniform suspension of very small particles distributed throughout the field. Under the high power ($\frac{1}{8}$ inch) the "negative" appears as a suspension of evenly distributed single small particles. Occasionally a few groups of 2 or more particles may be seen.

The "positive" under the low power shows large multiform flakes, which under the high power appear as clusters composed of from about 10 to 30 or more single particles.

For routine work only the low power objective is used, but in a very occasional doubtful case the high power lens may have to be employed.

Results obtained

Up to the 18th August 1944, a total of 2,581 specimens of serum and of cerebrospinal fluid were examined by the S.S. method. Of the above number 1,116 have been compared with the Kahn and Wassermann reactions performed in 2 independent outside laboratories. Of the 1,116 checked serum and cerebrospinal fluid specimens, 196 were S.S. positive and 920 S.S. negative.

In 61 out of the 1,116 checked specimens there was disagreement among the S.S., Kahn and Wassermann reactions, as under.

(1) Twenty-six specimens were S.S. negative and Wassermann negative, but Kahn positive. All these 26 specimens were taken from demonstrably non-syphilitic cases; in 8 cases the patients were girls who were about to be married and required a certificate; the Kahn test, repeated after a few days, gave a negative result in these cases. The remaining 18 cases showed no history of syphilis and no clinical symptoms, and blood was taken by the special clinic only as a precaution.

(2) Eight specimens were S.S. positive and Wassermann positive, but Kahn negative. All these 8 specimens were from patients with active syphilis under treatment at the special clinic.

(3) Four specimens were S.S. positive and Kahn positive, but Wassermann negative. These 4 specimens were from active cases of syphilis.

(4) Five specimens were S.S. negative and Kahn negative, but Wassermann positive. Four of the 5 cases concerned subsequently, without further antisyphilitic treatment and within a period of 8 weeks, gave negative Wassermann reactions. The fifth case showed a negative Wassermann reaction after a further short course of treatment.

(5) Two specimens were S.S. negative, but Kahn and Wassermann positive. These 2 cases are still undergoing treatment, and the latest tests have shown S.S. negative, Kahn doubtful and Wassermann doubtful.

(6) Sixteen specimens were S.S. positive, Kahn negative and Wassermann doubtful. Subsequent serological investigation by means of repeated Kahn and Wassermann reactions showed the S.S. results to be probably correct.

Considering the 23 cases enumerated under (4) (5) and (6), it seems possible that the S.S. technique shows reagin changes earlier than do the Kahn and Wassermann reactions. However, the number of cases is too small for generalization and further investigation is required.

Advantages of the method

It may be appropriate to mention here the advantages which the S.S. method appears to have over the Kahn and Wassermann reactions.

- (1) Venepuncture is not required.
- (2) The technique is simple and can be performed in any pathological laboratory.
- (3) A result can be obtained within a few minutes.
- (4) Use of this test eliminates the troublesome difficulty of serum being anti-complementary.
- (5) The test can be carried out on haemolysed serum.

Summary

A microscopical method for the serodiagnosis of syphilis is described, and the

advantages which it appears to have over the Kahn and Wassermann reactions are suggested.

REFERENCES

Rappaport, F., and Eichhorn, F. (1943) *Lancet*, 1, 426.

INVESTIGATIONS IN THE SERODIAGNOSIS OF SYPHILIS

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The need for a rapid and accurate test for the serodiagnosis of syphilis has led to the following investigations. The S.S. test, as described by Seiler, has been used at this clinic for 1,326 cases, which have been cross-checked by other serological reactions performed by outside laboratories. In all cases in which differences were found, blood investigations were repeated. Many sera were duplicated under different numbers. The antigen used in the S.S. test is that described by Rappaport and Eichhorn. All the tests carried out at this clinic were made in rotation and no effort was made to select cases.

Comparative results of tests

The results have shown a marked difference between the Kahn reaction and the S.S. test, but the S.S. tests have agreed to within 1 per cent with the Wassermann reaction. The percentage difference between these tests is still under investigation, the S.S. being regular in its results, the Wassermann reaction showing some irregularity. The percentage difference between the Kahn and S.S. tests remained constant through the whole period of the investigation, at about the 5 per cent figure.

Orpwood Price has called attention in a letter in the *Journal* to the fact that of late the reliability of the Kahn reaction has shown many variations, which may be due to a loss of sensitivity of the antigen.

Twenty-two cases sent up to this clinic as latent or suspected cases of syphilis, because of a positive Kahn reaction only, were found on full investigation to be non-venereal. The Wassermann and S.S. results in all these cases were persistently negative. In 18 of these cases the Kahn reverted to a negative reaction in spite of

TABLE—COMPARISON OF TESTS FOR SYPHILIS

TEST	AGREEMENT		DISAGREEMENT				
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
W. R.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Kahn	Pos.	Neg.	Neg.	Pos.	Neg.	Pos.	Pos.
S.S.	Pos.	Neg.	Pos.	Neg.	Neg.	Pos.	Neg.
Number of tests ...	182	1,072	10	48	7	2	5

the fact that no treatment was given. In the remaining 4 cases the Kahn test remained positive: of these cases, 3 exhibited a tuberculous pyrexia and one was possibly a case of acute rheumatism.

In cases of gonorrhoea in which a routine blood test for the serodiagnosis of syphilis was taken, 16 cases were returned as Kahn double plus, S.S. negative. Later, on fuller investigation, all these tests (controlled by the Wassermann reaction) were returned as negative.

Whenever a disagreement occurs between tests it has been found the S.S. test has been consistent in its results, whereas both the Kahn and Wassermann reactions